ephrin-A5, the neural crest cells are less likely to populate BA3 but the other branchial arches are not affected. We find that ectopic ephrin-A5 affects the direction in which the cells migrate from the neural tube. Our results suggest two ways in which ephrin signaling is involved in cranial neural crest migration. First it can act as a guidance cue. Second, it plays a role in regulating cell death and proliferation at varying steps of migration.

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Dorsal–ventral limb motor innervation choice in influenced by EphB/ephrin-B signaling

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Two groups of spinal cord motor neurons of the lateral motor column, the lateral (LMCl) and the medial (LMCm) subdivisions, innervate the dorsal limb and the ventral limb. While LMCl axons prefer the dorsal limb and their targeting is controlled by EphA/ephrin-A signaling, less is known about how LMCm axons are targeted to the ventral limb. To elucidate how LMCm axons are targeted, we asked whether their trajectories changed in mutants with limbs that lack ventral tissue. In the bidorsal limb conditional mouse mutant Brn4-creTg/−, BmprIaflox/− LMCm axons do not enter the limbs and are deflected to the ventral flank, while LMCl axons innervate both dorsal and dorsalized ventral limb tissue. Since in this mutant, motor neuron specification is not perturbed, yet limbs are bidorsal, these data show LMCm axons strongly prefer ventral over dorsal limb and have a relative preference for the ventral flank. Since EphA/ephrin-A signaling controls LMCl targeting, we investigated whether Eph/ephrin signaling also controls LMCm targeting. We found that EphB receptors are expressed in motor neurons. Repulsive ligand ephrin-B2 is expressed in the dorsal limb and is controlled by the dorsal limb determinant Lmx1b. Expression of EphB1 in all motor neurons redirects many LMCl axons to the ventral limb while the inactivation of EphB1 results in many LMCm axons projecting to the dorsal limb. Our results show that, in contrast to LMCl axons that have a preference for dorsal over ventral limb tissue, LMCm axons prefer ventral limb and that LMCm targeting is actively controlled by EphB/ephrin-B signaling.

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Regulation of ephrin-A5 during motor axon pathfinding to the hindlimb

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Motor axons project precisely from the neural tube to innervate their muscle targets in the chick hindlimb. We are interested in identifying the guidance cues that regulate axon behaviour during their extension and discerning how they work mechanistically. Previous studies from our lab have shown that EphA4 acts as a guidance cue for certain motor axons, directly them dorsally in the limb. Moreover, motor axons navigating to the base of the hindllimb express both EphA4 and its ligand ephrin-A5 and fasciculate in the spinal nerve. However, once motor axons reach the base of the limb, ephrin-A5 protein is lost on axon shafts and their growth cones, but ephrin-A5 transcript and protein remains localized to motor neuron cell bodies. Together, these observations raise the interesting questions of how ephrin-A5 is regulated on motor axons and whether ephrin-A5 downregulation is needed for motor axons to sort correctly into dorsal or ventral nerve trunks. The goals of my project are to test whether ephrin-A5 is required for spinal nerve fasciculation and whether maintenance of ephrin-A5 on motor axons disrupts axon sorting. To investigate the requirement for ephrin-A5 during axon fasciculation, we are utilising a new RNAi expression vector U14GFP-SIBR. This vector expresses shRNAs from an intron, using sequences derived from the miR-155 microRNA, as well as a GFP marker protein. Experiments are in progress to analyze whether selected ephrin-A5 shRNAs reduce ephrin-A5 protein in vitro and in vivo, prior to analyzing the effects of decreased ephrin-A5 protein on motor axon fasciculation.

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Ret and EphA4 signaling in motor axon pathfinding

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Motor neurons extend their axons precisely to innervate target muscles. This process involves a series of hierarchical axon guidance decisions by which motor neurons evaluate peripheral guidance cues and choose their axonal trajectory. Our previous studies have shown that EphA4 is necessary and sufficient for motor axons to project dorsally in the hindlimb. Recently, we showed that Ret and its ligand GDNF act in the same guidance decision, in contrast to their previously characterized functions in cell death and kidney development. In GDNF or Ret mutant mice, LMC(l) axons follow an aberrant ventral trajectory away from dorsal limb territory enriched in GDNF. This phenotype is enhanced in mutant mice lacking both Ret and EphA4. Ectopic expression of Ret in LMC(m) neurons in chick results in a subset of motor axons misprojecting dorsally instead of their normal ventral trajectory, as well as axon stalling at the presumptive sorting region at the limb base. Thus, EphA4 and Ret signals work together to enforce the precision of the same binary choice in motor axons. Our goal is to define exactly how EphA4/ephrins act with Ret/GDNF to drive axons dorsally

in the limb. Experiments are in progress to define the spatiotemporal pattern of expression of these factors in chick and to identify their distinct and cooperative functions during steps in motor axon pathfinding to the hindlimb.

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The role of B-class Eph receptors and ligands on midline guidance in the embryonic mouse spinal cord

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Ephrin and Eph receptors are known to regulate pathfinding at the midline of the central nervous system. In the embryonic spinal cord, ephrin-B3 is localized to the floor plate at the ventral midline (VM), while EphB receptors are expressed on decussated spinal commissural axons. Additionally, B-class ephrins are expressed in dorsal regions of the spinal cord, leading us to speculate that they may form a repulsive boundary that constrains the dorsoventral positioning of longitudinal commissural axons. Despite these compelling expression patterns, an *in vivo* role for EphB receptors/ephrins on the guidance of spinal commissural axons has yet to be established. Here, we used DiI labeling to assess the pathfinding of commissural axons in the spinal cords of ephrin-B and EphB mutants. In embryos lacking ephrin-B3 or multiple EphB receptors, a number of commissural axons exhibited guidance errors near the VM, compared to wild- type embryos. Furthermore, a particular commissural axon subtype, referred to as forked transverse commissural (FTC), which continues to project in the transverse plane on the contralateral side of the floor plate, was observed at a markedly higher frequency in ephrin-B3 and EphB mutant embryos. Interestingly, neither of these the midline guidance errors were apparent in the spinal cords of *ephrinB3*^{lacz} mice, which express a truncated form of ephrin-B3 that is incapable of reverse signaling. In contrast to the VM-associated phenotypes, we found that decussated commissural axons appear wild-type-like in B-class ephrin mutants.

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Dishevelled mediates ephrinB1 signalling in the eye field through the planar cell polarity pathway

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An important step in retinal development is the positioning of progenitors within the eye field where they receive the local environmental signals that will direct their ultimate fate. Recent evidence indicates that ephrinB1 functions in retinal progenitor movement, but the signalling pathway is unclear. We present evidence that ephrinB1 signals through its intracellular domain to control retinal progenitor movement into the eye field by interacting with Xenopus Dishevelled (Xdsh), and by using the planar cell polarity (PCP) pathway. Blocking Xdsh translation prevents retinal progeny from entering the eye field, similarly to the morpholino-mediated loss of ephrinB1. Overexpression of Xdsh can rescue the phenotype induced by loss of ephrinB1, and this rescue (as well as a physical association between Xdsh and ephrinB1) is completely dependent on the DEP (Dishevelled, Egl-10, Pleckstrin) domain of Xdsh. Similar gain- and loss-of-function experiments suggest that Xdsh associates with ephrinB1 and mediates ephrinB1 signalling through downstream members of the PCP pathway during eye field formation.

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Frazzled regulation of myosin at the *Drosophila* CNS midline

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Frazzled (Fra) is a key receptor in axon guidance. At the *Drosophila* embryonic midline Fra guides exploring neurons across developing commissures. It is likely to regulate actin and myosin dynamics and movement using a variety of signaling pathways. By reducing or over-expressing Fra in a subset of CNS neurons we provide genetic evidence that Fra initiates several signaling pathways to regulate myosin II activity. Over-expression of Fra enhances crossovers seen with constitutively active myosin light chain kinase (ctMLCK), while heterozygous fra mutations suppress ctMLCK-mediated crossovers. Interestingly, heterozygote mutations reducing Abelson tyrosine kinase (Abl) suppress these crossovers. Rho family GTPases, Rho, Rac and Cdc42, show ectopic crossovers when co-expressed with Fra; abl mutations alter these interactions. Rac and Cdc42 may regulate MLCK or myosin II thru p-21 activated kinase (Pak) or indirectly via regulation of actin dynamics. While Rho, through its major effector Rho kinase (Rok), alters myosin II activity by inhibiting myosin phosphatase via phosphorylation of its myosin binding subunit. We hypothesize that Fra regulates myosin dynamics at the midline through a Rho–Rok dependent pathway and actin dynamics through a Cdc42-Rac dependent pathway. We are testing this hypothesis using profilin and myosin loss-of- function alleles to tease apart the respective roles of actin versus myosin regulation downstream of Fra. Our work in deciphering how activation of Frazzled translates into forward movement of the